Breakthroughs Put the

Half a century ago, a potent combination of antibiotics, vaccines, and public health measures seemed poised to win the ancient war against infectious disease. Even malaria appeared to be succumbing to a mix of insecticides, larvicides (used to kill the mosquito vector), and drugs (used to kill the malaria-causing *Plasmodium* parasite in the human bloodstream). But while smallpox and polio were conquered, malaria was not; mosquito evolution and concerns about health effects from insecticides blunted mosquito eradication campaigns, and *Plasmodium* developed resistance to many drugs. Today, malaria is resurgent in many tropical regions, especially Africa. According to the World Health Organization, each year it infects more than 300 million people and kills at least 1 million, mostly children.

In the last year, however, major progress has been reported in basic research on malaria. One research group has reported a genetic manipulation that impairs the mosquito's ability to transmit the malaria parasite. Another has reported progress toward a vaccine that targets a newly discovered toxin made by *Plasmodium*. And this October, preliminary genomes were reported for the major malaria parasite, *P. falciparum*, and the major vector, the *Anopheles gambiae* mosquito.

An Antimalarial Mosquito

Human malaria is caused by four members of the genus *Plasmodium*, which goes through a complex life cycle. After a person is bitten by an infected mosquito, the parasite multiplies for a few days in the liver, and then is distributed through the blood. When other mosquitoes take a "blood meal" from an infected person, the parasite sexually reproduces in the mosquito's gut. The parasite leaves the gut and reaches the salivary glands, at which point the mosquito is poised to infect another person.

The first symptoms of malaria start when *Plasmodium* enters the blood, when the characteristic "paroxysms"—cycles of fevers and chills—begin. Malaria may also cause renal or pulmonary failure; cerebral malaria, which usually afflicts children and pregnant women, can cause coma, generalized convulsions, and death.

Whereas malaria fighters have historically attacked the parasite or the vector, other targets are emerging from the study of the complicated interaction of parasite, vector, host, and environment. Many mosquito species, for example, are resistant to infection with *Plasmodium*. Could that resistance be transferred to those that do transmit malaria? In the 23 May 2002 issue of *Nature*, Marcelo Jacobs-Lorena, a professor of genetics at Case Western Reserve University, and colleagues reported on a major advance toward answering that question—they genetically engineered a strain of mosquito with an impaired ability to spread a rodent malaria parasite.

The researchers focused on receptor molecules on the epithelium of the mosquito gut. During the ookinete life stage, the parasite links to these receptors as it migrates to the salivary glands. The researchers made about 1 billion artificial peptides and found one that bonded to the gut's lining, blocking receptors there. Because the blood meal is the point at which the mosquito becomes infected with *Plasmodium,* any gene activated when the blood is digested could possibly also be pressed into service as a malaria fighter. So the team inserted a gene for this peptide into the mosquito genome, and instructed the genome to activate the gene after the mosquito took a blood meal.

Targeting a toxin. A new malaria vaccine targets a compound made by *Plasmodium* that may be the source of malarial pathology.

Only two of the three groups of modified mosquitoes actually failed to transmit malaria, says Jacobs-Lorena. He adds that the peptide is not 100% effective; there are always escapees. "That's one of many reasons why this is just a first step in the right direction," he says. Still, the experiments reported in *Nature* were proof in principle that it's possible to block the spread of malaria, he says.

Many questions about this application of transgenic technology remain to be answered. One concerns testing efficacy and safety. Bruce Christensen, a professor of animal health and biomedical sciences who studies the interaction of malaria parasites and mosquito vectors at the University of Wisconsin at Madison, says, "It's extremely difficult . . . to see how the variety of different [parasite] genotypes respond in the engineered mosquito. Whenever you are dealing with a pathogen–host relationship, it's a two-way street. The presence of one influences the gene expression in the other. . . . To thoroughly understand the relationship is brutally difficult research to do."

The malaria-resistant mosquitoes must also outcompete normal malaria-vectoring

Left: Image Source/elektraVision/PictureQuest; facing page: Jim Gathany/Centers for Disease Control and Prevention

Bite on Malaria

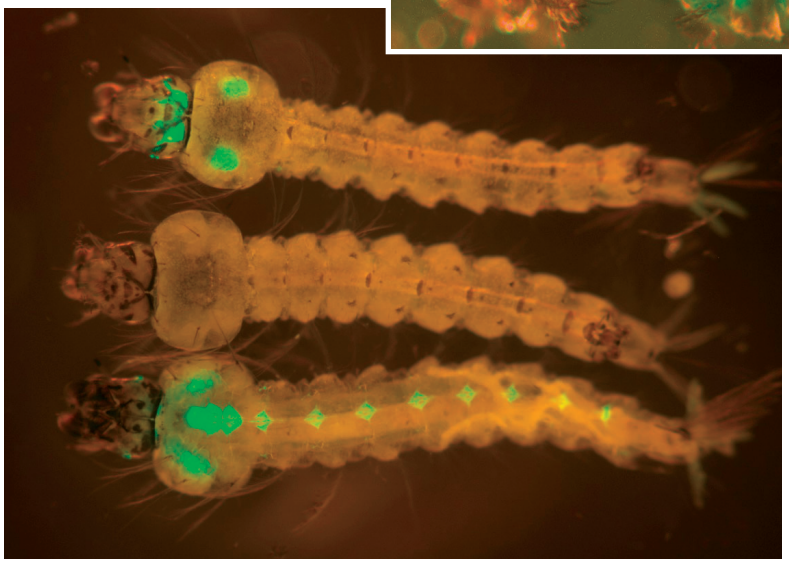
mosquitoes—they must be more genetically fit. Richard Carter, a professorial fellow in the Division of Biological Sciences at Scotland's University of Edinburgh, says the effect on fitness depends upon the specific genetic modification involved. In the case of Jacobs-Lorena's work, he says, "the modification might have some chance of becoming established in wild mosquito populations if it carries very little intrinsic cost to the mosquitoes [and] otherwise makes them healthier, because, unlike wild mosquitoes, they do not risk becoming infected with malaria." However, Carter admits that research on whether freedom from malarial infection can be expected to cause mosquitoes to live longer or reproduce better has produced "rather a mixed bag of results."

Any antimalaria strategy must survive the parasite's proven ability to evolve and thus evade control strategies. In this respect, Carter thinks Jacobs-Lorena's technique has an advantage. "At [the ookinete] point in their life cycle, the parasites are not in fact increasing in numbers, but decreasing," he says. "Two gametes form a zygote—one cell from two—and the zygotes of malaria parasites do not multiply in the midgut. Thus, in terms of the possibility of selection, we are talking tiny numbers; only one to a few dozen zygotes are destined to survive to form oocysts in a single mosquito under any circumstances."

Conversely, he says, conventional malaria drugs attack billions to trillions of parasites that are multiplying in the human bloodstream, which he calls a "classic situation for selecting for resistant microorganisms." Because a much smaller number of nonmultiplying parasites exists in the mosquito's gut, he says, "the selection pressure is orders of magnitude smaller."

However, numerous practical obstacles remain before a successful release of genetically modified mosquitoes can be expected. Carter observes, for example, that modified insects have generally failed to control disease. The sole exception was a campaign against the screwworm, a cattle pest in the southern United States, in which the release of millions of sterile adult male screwworm flies caused massive breeding failure among the pest. The screwworm was declared eradicated from the United States in 1966.

The environmental impact would also need careful study, according to a group of ecologists who discussed the matter in the 5 July 2002 issue of *Science*. Politics and public education are major areas of concern. For example, people who have been told to protect themselves against mosquitoes might well question the release of millions of additional mosquitoes. Christensen notes that a mosquito-control project designed to release millions of sterile male mosquitoes in India several decades ago infuriated the intended beneficiaries: "People burned the trucks and chased the public health officials away."



You know by the glow. Mosquitoes that have been genetically manipulated to impair their ability to transmit malaria glow with a fluorescent green marker (inset: right; large picture: top and bottom).

Aggravating the situation would be the fact that the mosquitoes would be genetically engineered. Recalling the recent uproar in Zambia over the safety of genetically engineered corn donated to feed starving people, Joseph Vinetz, a malaria researcher who is an assistant professor in the Center for Tropical Medicine at the University of Texas Medical Branch in Galveston, asks, "If we could release an environmentally friendly mosquito that is refractory to malaria, who is going to believe it's harmless?"

Still, Vinetz argues, solutions to malaria must be judged according to the severity of the problem. "Malaria is one of the most important problems in the entire world," he says. "Therefore, the threshold for doing something [to address it] should be lower than for dealing with something less important."

Vaccine Technology

The year 2002 also saw progress toward a novel malaria vaccine technology, described in the 15 August 2002 issue of *Nature*. In contrast to most existing vaccines, the new vaccine targets not proteins on the pathogen's exterior but rather a compound called glycosylphosphatidylinositol (GPI) that seems to cause malaria's most deadly symptoms—and may be a long-sought malaria toxin. Antitoxin vaccines are effective against tetanus and diphtheria.

The research grew from frustration with the conventional approach to malaria vaccines, says Louis Schofield, first author of the August *Nature* paper. Schofield, a researcher at the Walter and Eliza Hall Institute of Medical Research in Melbourne, Australia, says that when he did his Ph.D. work in the 1980s, "there was great enthusiasm for developing a malaria vaccine, and the whole world was busy cloning parasite surface proteins." Schofield, however, worried that proteins that did not interact with the human host would be susceptible to the Achilles' heel of malaria vaccines—the genetic variability of the pathogen. In other words, if such proteins were not intimately involved in the disease process, they would not be conserved by evolution, and thus might be so variable as to be useless as drug targets.

Instead, Schofield was intrigued by GPI. Various GPIs, Schofield says, tether proteins to the cell surface in all multicelled organisms. Schofield found that *Plasmodium* GPI was associated with a complex lipid known as diacylglycerol, a molecule that regulates a variety of cellular functions and is known to activate protein kinase C. He also found that *Plasmodium* GPI would hyperactivate macrophages and antigen-presenting cells in the immune system, and cause red blood cells to adhere to blood vessels—all conditions

Jacobs-Lorena laboratory

associated with deadly cerebral malaria. "At last we had a parasite molecule that could possibly cause the widespread host cell activation that causes pathology in malaria," he says.

GPI, in other words, looked like a toxin. Schofield spent years examining whether this was actually the case in cell culture and animal research, but too little of the compound was available to really test the proposition. Eventually, Peter Seeberger, a chemist at the Massachusetts Institute of Technology, synthesized a larger quantity of pure *Plasmodium* GPI, which was used in the experiment reported this year in *Nature*. "[Schofield] had a feeling that it might be the toxin for a long time," says Seeberger, "but if you purify such a tiny amount, you can't characterize it. An impurity may be responsible for the toxicity."

When Schofield and colleagues vaccinated mice with the pure, synthesized *Plasmodium* GPI, it prevented most of the symptoms of cerebral malaria and reduced acidosis (elevated blood acidity), a condition closely linked to mortality in malaria. The GPI vaccine works by stimulating the production of antibodies that, as Schofield puts it, "latch on to the toxin and inactivate it."

As the vaccine work moves toward animal efficacy and toxicity research, several questions remain open. Although malaria eventually killed the experimental mice, Schofield attributes that to a massive growth of the parasite that occurs in mice but not in humans, and thus he thinks it should not be a cause for concern. Another unknown is how long the antibody will continue to neutralize the toxin. "I don't think any antibody–antigen bond is irreversible," Schofield says, "but if [it lasts] sufficiently long to block the action of the toxin, that's long enough."

A more basic uncertainty concerns the status of GPI. Is it the principal malaria toxin? The only one? "Schofield was very careful about this," says Thomas Richie, director of malaria clinical trials at the Naval Medical Research Center in Silver Spring, Maryland. "*Plasmodium* is a complex parasite, and in general, many of its key interactions with the host may be mediated by more than one molecule. What we have learned about the pathophysiology of severe malaria in humans suggests that GPI by itself is unlikely to be the sole mediator of clinical illness." Still, in a research field that's perennially short on good news, Richie says the GPI vaccine is "a great development."

Genomes Galore

In October, two large international collaborations marked milestones in the war against malaria: publication of the genome for *P. falciparum* in the 3 October 2002 issue of *Nature* and the genome for *A. gambiae* in the 4 October 2002 issue of *Science*. The research reports, together with numerous associated articles, describe the genomes and suggest how the new information might be used to control malaria.

The mosquito genome, described by a group under the leadership of Robert Holt of Celera Genomics in Rockville, Maryland, contains 278 million base pairs—about 10% as large as the human genome. In addition to publishing the DNA sequence, Holt's group focused on a topic of considerable interest among malaria researchers: the genetic effects of the mosquito's blood meal. While the female is digesting mammalian blood, genes for protein and lipid metabolism are up-regulated, while genes for muscular and sensory activities are down-regulated.

Knowledge of the mosquito genome may change the way insecticides are developed. Rather than testing them against the whole insect, they may be tested against gene products. Indeed, Holt says development of insecticides and vaccines may be the most important first results of the genome work: "I think the most important thing the genome will facilitate in the immediate future is understanding the molecular basis for resistance to insecticides and finding new insecticide targets." Another avenue for study would be to genetically engineer the mosquito so it would be less attracted to humans as a source of blood.

With significant scientific synchrony, Malcolm Gardner of the Institute for Genomic Research in Rockville and colleagues published the *P. falciparum* genome the same week. The sequencing team tentatively identified 5,279 genes, but said the function of 60% were unknown. The parasite is genetically distinct from all previously sequenced organisms, which makes the task of taking advantage of the genome more difficult.

Russell Doolittle, a research professor with the Center for Molecular Genetics at the University of California at San Diego, observes that the parasite genome offers "almost too many options to pursue" in terms of new targets for drug treatment. One group of targets identified by the genome authors were protein-degrading enzymes found in a subcellular digestive component called the food vacuole. These enzymes are involved in digestion of the blood meal.

However, Doolittle also warned in a commentary in the 3 October 2002 *Nature* that the genomes may promise more than they can deliver in terms of actually reducing the burden of disease. Noting a continual tension between on-the-ground measures to control malaria (such as bed nets and larvicide spraying) and high-tech approaches (such as vaccines and malaria-resistant mosquitoes), he asked, "So is it worth it from a medical point of view? That really remains to be seen." Whole-genome projects, he wrote, have delivered more to basic biology than to medicine; for the most part, the promised medical benefits have been slow to materialize.

It's impossible to say whether or when these advances against malaria will bear fruit by reducing the burden of disease. But malaria researchers already know from long experience that their enemy is devious and resourceful, and the dominant sentiment in the field seems to be a preference for hedging bets. Jacobs-Lorena, for example, says, "I can't imagine that the transgenic mosquito would be one hundred percent effective. There's a strong consensus in the field that only a combination of approaches will really work to solve the problem."

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Suggested Reading

Enserink M. 2002. Malaria. Ecologists see flaws in transgenic mosquito. Science 297(5578):30–31.

Ito J, Ghosh A, Moreira LA, Wimmer EA, Jacobs-Lorena M. 2002. Transgenic anopheline mosquitoes impaired in transmission of a malaria parasite. Nature 417(6887):452–455.

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Schofield L, Hewitt MC, Evans K, Siomos MA, Seeberger PH. 2002. Synthetic GPI as a candidate anti-toxic vaccine in a model of malaria. Nature 418(6899):785–789.